Original Article

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Seroprevalence of three influenza A viruses (H1N1, H3N2, and H3N8) in pet dogs presented to a veterinary hospital in Ohio

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The prevalence of canine H3N8 influenza and human H1N1 and H3N2 influenza in dogs in Ohio was estimated by conducting serologic tests on 1,082 canine serum samples. In addition, risk factors, such as health status and age were examined. The prevalences of human H1N1, H3N2, and canine H3N8 influenzas were 4.0%, 2.4%, and 2.3%, respectively. Two samples were seropositive for two subtypes (H1N1 and H3N2; H1N1 and canine influenza virus [CIV] H3N8). Compared to healthy dogs, dogs with respiratory signs were 5.795 times more likely to be seropositive against H1N1 virus ($p = 0.042$). The prevalence of human flu infection increased with dog age and varied by serum collection month. The commercial enzyme-linked immunosorbent assay used in this study did not detect nucleoprotein-specific antibodies from many hemagglutination inhibition positive sera, which indicates a need for the development and validation of rapid tests for influenza screening in canine populations. In summary, we observed low exposure of dogs to CIV and human influenza viruses in Ohio but identified potential risk factors for consideration in future investigations. Our findings support the need for establishment of reliable diagnostic standards for serologic detection of influenza infection in canine species.

Keywords: canine influenza, cross-sectional studies, prevalence, risk factors, serology

Introduction

Type A influenza virus has been identified in human and diverse animal species [35]. The host range of specific influenza viruses is usually restricted to a few species, but their continuous genetic evolution enables them to adapt to new species [13,20,34].

The first lineage identified as successfully established in dogs is the canine influenza virus H3N8 (CIV H3N8), initially isolated from racing greyhound dogs in 2004 in the USA [7]. CIV H3N8 incidence has been sporadically reported across the USA and has become enzootic in New York, Colorado, and Florida [4,5,12,15,25]. Dogs are the only known natural host of CIV H3N8, but the virus shares monophyletic origin with an equine lineage, which has been circulating among horses in the USA since the 1990s [7,25]. Another canine specific lineage, the CIV H3N2, was isolated first from Korean dogs in 2007 [28]. Sequence analysis showed that CIV H3N2 originated from an avian lineage without reassortment [28]. The clinical signs caused by CIV H3N2 can be reproduced in diverse mammalian species, including dogs, cats, ferrets, and guinea pigs [17,22]. It was previously assumed that CIV H3N2 was restricted to East Asian countries, but, in April 2015, a large cluster of CIV H3N2 cases emerged in the Midwestern USA (Centers for Disease Control and Prevention, USA). More than 1,000 dogs in the Chicago, IL area were confirmed or presumptively infected with CIV H3N2, and it was rapidly spread to other Midwestern areas (Cornell University College of Veterinary Medicine, USA). It is unclear how the virus traveled across continents and how the virus could quickly spread within the USA.

The identification of a canine-adapted lineage in the 21st

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century is rather surprising because it has been thought that canine species does not play a major role in influenza epidemiology, despite their frequent contacts with human and avian species [11]. Little has been reported about how the lineage successfully adapted in canine species [7,25] or about how the adapted lineage could spread across geographically remote areas [25]. Several studies have tried to identify the infection status and risk factors, such as exposure to animal shelters or dog daycare centers [3,5,14]. However, these studies primarily concentrated on specific areas with relatively high CIV incidence and were conducted using a variety of diagnostic methods, which may not provide information on influenza status in the general canine population.

Although canids have been neglected regarding their potential role as a reservoir of influenza virus, influenza infection has been described in dogs after massive exposure to virus during pandemics or consumption of influenza-infected carcasses [10,23,30]. A serologic study conducted on canine sera in China suggested continuous exposure to human H3N2 in dogs [31]. In addition, a natural reassortment strain between pandemic H1N1 2009 and CIV H3N2 was identified in dogs from Korea [29]. Even though there are no reports of dog-to-human transmission, it is prudent to be alert to the possible emergence of novel strains with zoonotic risk in the canine population.

This study evaluated the exposure status and potential risk factors of both canine and human influenza in dogs by testing serum samples collected in Ohio during 2012 to 2014. We used three different conventional serologic assays for which diagnostic performance has not yet been established in dogs.

Materials and Methods

Sample collection and viruses

Sera (n = 1,082) were passively collected by using excess material from clinical samples derived from pet dogs presented to The Ohio State University Veterinary Medical Center between October 2012 and June 2014. Most samples (n = 924) were sourced from patients presenting with complaints unrelated to the respiratory system, such as orthopedic procedures, cancers, congenital malformations, endocrinopathies, and neurologic diseases. Forty-seven samples were derived from individuals with a primary complaint attributable to the respiratory system. The remaining 111 samples came from animals presented for wellness care, dental procedure, and accidental physical trauma, which were categorized as healthy for our analysis. As controls, serum samples collected from laboratory dogs experimentally infected with CIV H3N8 were used. As antigens for the subtype-specific hemagglutination inhibition (HI) test, H1N1 (human/OH/0925-1/09), H3N2 (human/OH/182/06), and CIV H3N8 (A/canine/PA/94930-1/2007) were used. The canine (H3N8) and human (H1N1 and H3N2) viruses were kindly provided by Erica Spackman at the Southeast Poultry Research Laboratory, USDA (Athens, GA, USA) and Kathy Smith at the Ohio Department of Health (Columbus, OH, USA), respectively. Sera collected from uninfected specific-pathogen-free (SPF) dogs were used for negative controls, while sera collected from SPF dogs 15 days post-infection (dpi) with CIV H3N8 were used as positive controls in the HI tests. Reference sera used as positive and negative controls were kindly provided by Gabriele Landolt at Colorado State University (Fort Collins, CO, USA).

Hemagglutination inhibition test

The HI titer of canine sera was determined as previously described by using 96-well polystyrene round bottom microwell plates (Greiner Bio-One, USA) [1,32]. Briefly, all serum samples were heat inactivated at 56°C for 30 min. Two-fold diluted serum (50 μL) and the same volume of 8 hemagglutination (HA) unit virus of each subtype were mixed and incubated for 30 min at room temperature, followed by addition of 50 μL of 1% turkey erythrocyte suspension. Turkey red blood cells (RBCs) were used based on a previous study that demonstrated highest hemagglutination ability using turkey RBC compared to other RBCs with canine sera. The HI titer was reciprocally determined at the end-point dilution that showed complete HI, and a HI titer $\geq$ 1:16 was used as the cut-off value for seropositive samples. All HI-positive samples were treated with receptor-destroying enzyme (Denka Seiken, Japan) and retested to confirm potential false positive results due to non-specific reactions.

Virus neutralization test

Among HI positive serum samples, eleven samples for H1N1 and nine samples for H3N2 and H3N8, respectively, were selected for virus neutralization (VN) testing to confirm the HI test results. The procedure was conducted as previously described [1,32] with slight modification. Briefly, 25 μL of two-fold serially diluted serum was incubated with the 100 $TCID_{50}$ viruses for 1 h at 37°C. The serum-virus mixture was then transferred to MDCK cells in 96-well cell culture plates and after 24 h, the supernatant replaced with fresh serum-free media. The plates were observed for 4 days for cytopathic effects (CPEs), such as aggregation and detachment of cells. The VN titer was the reciprocal of the highest dilution that completely inhibited CPE formation. The neutralization result was confirmed by the absence of hemagglutination activity in cell culture media supernatants after incubating 50 μL of supernatant with 50 μL of 1% turkey erythrocyte.

Detection of influenza A nucleoprotein-specific antibody by competitive ELISA

Samples were tested by using a competitive enzyme-linked immunosorbent assay (cELISA) kit (Influenza A NP antibody

inhibitor ELISA; Virusys, USA) and comparing its results with the HI results. All procedures were conducted according to manufacturer's instructions and the nucleoprotein (NP) reduction index (NPRI) value was calculated from the formula: NPRI = 1 − (absorbance value [Abs] of samples − mean Abs of diluent control)/(mean Abs of negative serum control − mean Abs value of diluent control). An NPRI $\geq$ 30 was considered to represent the presence of NP antibody in serum, while 30 > NPRI $\geq$ 20 and NPRI < 20 were considered 'suspect' and 'negative', respectively. For direct comparison of ELISA sensitivity with HI assay, the control positive samples with an NPRI value higher than 30 were serially diluted and tested again by cELISA to determine the detection limit.

Statistical analysis

To test for correlations between risk factors and seropositivity, a two-sided Pearson's chi-square test was used and a multinomial logistic model was constructed. The risk factors evaluated in this study included age (grouped as 0 < age < 2 years old, 2 $\leq$ age < 14 years old, and age $\geq$ 14 years old), health status (grouped as healthy, respiratory illness, and non-respiratory illness involved), and the month in which the serum samples were collected. The Spearman rank correlation coefficient was calculated to test the correlation between HI and VN test results. All statistical analyses were conducted by using IBM SPSS Statistics (ver. 22; IBM, USA) with statistical significance set at $p < 0.05$.

Results

Prevalence of three influenza viruses and correlation with risk factors

The prevalence of each influenza subtype was estimated by using HI tests (Table 1). Among 1,082 sera samples tested, 92 samples were positive against at least one subtype. Two samples were positive for two subtypes; one to human H1N1 and CIV H3N8, the other to human H1N1 and H3N2. No samples were HI-positive to all three subtypes. The median (range) HI titers of the positive samples for H1N1, H3N2, and H3N8 were 48 (16–256), 32 (16–128), and 32 (16–256), respectively.

Table 1. Prevalences of seasonal H1N1, H3N2, and canine H3N8 influenza in dogs presented to a veterinary hospital

Variable	H1N1	H3N2	H3N8	Total†
	4.0 (43/1,082)	2.4 (26/1,082)	2.3 (25/1,082)	8.5 (92/1,082)
Health status				
Healthy	1.8 (2/111)	0 (0/111)	4.5 (5/111)	6.3 (7/111)
Respiratory illness	10.6 (5/47)	4.3 (2/47)	2.1 (1/47)	17.0 (8/47)
Non-respiratory illness	3.9 (36/924)	2.6 (24/924)	2.1 (19/924)	8.3 (77/924)
p value	0.032*	0.168	0.267	0.078
Age (yr)				
0 $\leq$ age < 2	0 (0/65)	0 (0/65)	0 (0/65)	0 (0/65)
2 $\leq$ age < 14	4.0 (39/967)	2.4 (23/967)	2.6 (25/967)	8.8 (85/967)
14 $\leq$ age	8.0 (4/50)	6.0 (3/50)	0 (0/50)	14.0 (7/50)
p value	0.090	0.113	0.218	0.018*
Collection month				
Jan	2.0 (2/100)	2.0 (2/100)	0 (0/100)	4.0 (4/100)
Feb	2.1 (2/96)	0 (0/96)	1.0 (1/96)	3.1 (3/96)
Mar	5.4 (5/92)	2.2 (2/92)	0 (0/92)	7.6 (7/92)
Apr	9.1 (9/99)	1.0 (1/99)	6.1 (6/99)	14.1 (14/99)
May	1.9 (2/105)	1.9 (2/105)	1.9 (2/105)	5.7 (6/105)
Jun	5.7 (6/106)	3.8 (4/106)	3.8 (4/106)	13.2 (14/106)
Jul	7.7 (3/39)	0 (0/39)	5.1 (2/39)	17.9 (7/39)
Aug	5.4 (3/56)	0 (0/56)	3.6 (2/56)	8.9 (5/56)
Sep	0 (0/64)	14.1 (9/64)	4.7 (3/64)	18.8 (12/64)
Oct	1.7 (2/120)	0 (0/120)	0.8 (1/120)	2.5 (3/120)
Nov	7.1 (7/99)	2.0 (2/99)	1.0 (1/99)	10.1 (10/99)
Dec	1.9 (2/106)	3.8 (4/106)	2.8 (3/106)	8.5 (9/106)
p value	0.034*	< 0.001*	0.085	0.002*

Data are presented as % (number of seropositive samples/total number of samples). *P* value by two-sided Pearson's Chi-square test. *$p < 0.05$. †Total means seropositive to any subtype.

Prevalence was analyzed according to risk factors, and statistical differences were primarily tested by using two-sided Pearson's chi-square test (Table 1). When analyzed by health status, the prevalence of human strains was highest in the respiratory illness involved group and a significant difference was observed in the prevalence of subtype H1N1 ($p = 0.032$). A correlation between H1N1 prevalence and respiratory illness was also observed in the results of the multinomial logistic regression model analysis, which showed that, when comparing the healthy and respiratory illness groups, the risk of having an antibody against human H1N1 was 5.795 times higher in dogs with respiratory illness than in healthy dogs ($p = 0.042$) (Table 2). Regarding CIV H3N8, there were no significant differences in prevalence among the health status groups. All sera from dogs younger than 2 years old were negative, even though young animals are thought to be vulnerable to opportunistic infection in the respiratory tract [7]. In age group $2 \leq$ age < 14 years old, the prevalences of the three subtypes were similar with the overall prevalence of each subtypes. The prevalence of the human strain was observed highest in dogs older than 14 years old, while seropositivity of CIV H3N8 was not detected in the same age group. Statistical significance of differences was observed only when the three subtypes were considered altogether (Table 1), and no significant correlation was detected between age and any subtype of influenza in the multinomial logistic regression analysis. The seasonality of subtypes in sera was verified by testing groups according to the serum collection month. A significant difference by month was observed in prevalence of human H1N1 and H3N2 viruses. The seropositivity of H1N1 was higher than the overall prevalence (4.0%) in March, April, June, July, August, and November. For H3N2, the highest prevalence (14.1%) was observed in September 2013.

Table 2. Results of multinomial logistic regression analysis of correlation between health status and H1N1 seropositivity

Variable	Respiratory illness involved		Non-respiratory illness involved	
	Odds ratio (95% CI)	*p* value	Odds ratio (95% CI)	*p* value
Prevalence for H1N1	5.795 (1.068–31.345)	0.042*	2.137 (0.506–9.020)	0.301

CI, confidence interval. *$p < 0.05$.

Table 3. Detection of anti-influenza nucleoprotein (NP) antibody from hemagglutination inhibition (HI)-positive samples by commercial competitive enzyme-linked immunosorbent assay (cELISA)

HI titer		Total	cELISA			
			Negative (n)	Suspect (n)	Positive (n)	Positivity (%)
H1N1	8	14	12	2	0	0
	16	11	7	0	4	36.4
	32	9	4	1	4	44.4
	64	13	8	0	5	38.5
	128	8	1	0	7	87.5
	256	2	0	0	2	100
H3N2	8	37	35	1	1	2.7
	16	11	7	1	3	27.3
	32	7	6	0	1	14.3
	64	8	4	1	3	37.5
H3N8	8	5	5	0	0	0
	16	10	2	3	4	40.0
	32	5	3	0	2	40.0
	64	3	1	0	2	66.7
	128	3	0	1	2	66.7
	256	4	0	0	4	100

Data are presented as number of samples tested. Negative, suspect, and positive results were determined based on NP reduction index (NPRI) value < 20, $20 \leq$ NPRI value < 30, and $30 \leq$ NPRI value, respectively. Positivity (%) = percentage of samples with specific HI titer (8–256) of each subtype (H1N1, H3N2, and H3N8) $\times$ 100.

Table 4. Comparison of hemagglutination inhibition (HI) test and commercial competitive enzyme-linked immunosorbent assay (cELISA) results on positive serum samples obtained from experimentally infected dogs with canine influenza virus (CIV) H3N8

Sample*	Days post-infection	HI test result (end point dilution)†	cELISA	
			Test result (NPRI)‡	End point dilution
K1	Not infected	Neg (-)	Neg (1.152)	-
QZV	19	Pos (1:712)	Pos (89.10)	1:128
OUV	19	Pos (1:356)	Pos (91.66)	1:512
DFS	19	Pos (1:160)	Pos (40.36)	1:16
SZV	19	Pos (1:2,848)	Pos (90.66)	1:256
KKV	19	Pos (1:712)	Pos (90.56)	1:256

Neg, negative; Pos, positive. *Serum samples collected from dogs experimentally infected with CIV H3N8. †HI titer (end point dilution) ≥ 1:16 was considered as HI test positive. ‡Nucleoprotein (NP) reduction index (NPRI) value was determined and a NPRI ≥ 30 was considered cELISA positive, as described in the manufacturer's protocol; NPRI (%) = [1 − (sample Abs_{450} − diluent Abs_{450})/(negative Abs_{450} − diluent Abs_{450})] × 100.

Comparison of HI, VN, and cELISA test results

The VN test included HI-positive samples (HI titer > 16 units) randomly selected from each subtype (total 29 samples). The median (range) HI titers of the selected samples were 64 (16–256) for H1N1, 16 (16–256) for H3N8, and 16 (16–128) for H3N2. All selected samples showed neutralization activity, except for two samples from CIV H3N8 and one sample from H3N2. The median VN titer (range) was 90.5 (4–256) for H1N1, 32 (4–256) for CIV H3N8, and 16 (2–181) for H3N2. No cross-reactivity was observed among subtypes, and two parameters showed significant correlation ($p < 0.01$) in the Spearman rank correlation test results ($p = 0.001$, correlation coefficient = 0.804 for H1N1; $p < 0.0001$, correlation coefficient = 0.934 for H3N8; and $p = 0.007$, correlation coefficient = 0.708 for H3N2).

When we tested the HI-positive samples with the commercial cELISA kit, relatively low positivity was observed. As shown in Table 3, cELISA could detect anti-influenza NP antibodies in all six samples whose HI titer was 256. However, the detection ability of cELISA decreased as the HI titer went down, and only 1 of the 56 samples with a HI titer of 8 was determined to be positive by cELISA (Table 3), indicating the poor sensitivity of cELISA for canine serum.

The cELISA and HI test results were further compared by using CIV positive samples collected from experimentally infected dogs. The sera from dogs infected with CIV H3N8 were collected at 19 dpi (Table 4). The HI test detected CIV H3N8 specific antibody in all 5 serum samples collected at 19 dpi, but did not detect it in the non-infected negative control samples (Table 4). For a direct comparison of the sensitivity of each test method, the endpoint dilution was determined by cELISA testing of serially diluted positive samples. Except for one sample (OUV; Table 4), the HI test detected lower antibody titers than those detected by cELISA, which is consistent with the results described above.

Discussion

Despite a decade of research on CIV H3N8, little has been reported about its mode of transmission, and an emergency protocol for an outbreak in the canine population, as was observed in 2015 for CIV H3N2, is not available. This study was conducted to provide information about the prevalence of influenza in the dog population of Ohio, an area that has not received as extensive attention as that in canine influenza endemic areas.

Several tests are available for the diagnosis and surveillance of animal influenza [6]. Virologic methods are preferred for the diagnosis of acute infection, while serologic testing is essential for epidemiological studies to detect asymptomatic infections during outbreak investigations [18]. In this study, we used HI, VN, and ELISA tests, all of which are frequently used for influenza surveillance [18]. Both VN and HI assays are highly specific and sensitive for detecting strain-specific antibodies. Those two methods usually show correlated titers, but the HI assay is more widely used than the VN test due to its simpler procedure [32]. The ELISA test shows high sensitivity and has advantages in screening large number of samples in a non-subtype specific manner [8,10,19,26,33]. In this study, the cELISA results showed a HI-titer-correlated positive ratio and accurately detected samples with high HI titer (> 256). However, when we compared HI and cELISA test methods by direct comparison of the endpoint dilution on CIV H3N8 positive control samples, cELISA had a lower analytical sensitivity than that of the HI test (Table 4). Since we tested only one commercial cELISA, it should not be concluded that cELISA is generally less sensitive than the HI test in detecting low concentrations of antibody in canine sera. However, the accuracy and precision of commercial ELISA kits for surveillance of influenza in canine populations should be further validated.

Based on the HI test results, the prevalence of CIV H3N8 was 2.4%, which is similar to the prevalence previously observed in a general dog population (3.6%) [5]. Much higher prevalences (45%–58%) have been observed in specific populations, such as in racing greyhound dogs [7], dogs in animal shelters [14], and dogs in influenza endemic areas, such as Florida, Colorado, and New York, where the endemic CIV strain has been continuously confirmed [4,25]. However, it should be noted that the equine influenza virus (EIV) H3N8 can infect dogs and using the current serologic assays, we cannot distinguish the CIV from the EIV infection. Understanding the factors which contribute to viral exposure among different populations is essential when developing control measures against transmission of influenza among dogs and other species. Considering that direct contact with the virus is the major mode of transmission, one logical risk factor is the high frequency of contact with other dogs. However, in earlier study, only 3% of dogs in a "fly ball" tournament (a competitive canine sport) were positive, despite those dogs having a high frequency of contact with other dogs [27]. In the same study, a history of visits to endemic areas was not correlated with prevalence of CIV. Another study conducted on a general pet dog population in Colorado showed a significant correlation of CIV prevalence with involvement in dog daycare centers but not with other factors, such as visiting other countries or animal shelter exposure [5]. Seroprevalence of CIV H3N8 is affected by a combination of variables, such as exposure setting and geographic location [4]. It appears that the CIV H3N8 is now circulating in particular exposure settings, such as shelter facilities, and in geographically limited regions [24]. Taken together, those findings suggest that the prevalence of CIV in the general population is maintained at a low level.

Despite the frequent interaction between humans and dogs, the prevalences of human H1N1 and H3N2 (4.0% and 2.6%, respectively) were similar to that of CIV H3N8 (2.3%). A recent study conducted in China also showed low prevalences of H1N1/09 (1.5%) and human H3N2 (1.2%) in dogs [31]. In addition, a previous challenge study in dogs with H1N1/09 virus showed that the virus could replicate in the canine respiratory system but was not able to transmit the virus through contact with control dogs [20]. Based on those results, although human influenza virus is capable of infecting dogs, the virus replication efficiency may be low and insufficient for infection to result in detectable seroconversion.

Even though human influenza virus transmission efficiency among canine population appears to be low, the zoonotic importance of canine influenza should not be overlooked. A recent report raised a concern about the co-infection of two different viruses in dogs and the risk of chimeric virus generation [29]. That report identified a novel H3N1 CIV in dogs, which appeared to be derived from a recombination event between human and canine viruses. This virus replicated in canine respiratory tract and caused mild histopathologic lesions in the experimentally infected dogs [29]. In this study, two serum samples harbored antibodies against two different subtypes. Since a serum antibody can last for months, we cannot be sure whether two different viruses were co-infected at the same time or if we detected two subtypes of antibodies that were sequentially developed. Regardless, our finding suggests that there could be a chance of co-infection of influenza viruses derived from two different hosts, which can result in generation of a novel strain.

One of the risk factors analyzed in this study was the health status of the dog. The serologic data for CIV did not show any significant correlation with disease status, which is consistent with a previous report [5]. The reason that pathogenicity of CIV H3N8 is not reflected in our results or in previous studies may be due to the sera being collected passively, and dogs with infectious respiratory disease were thus not targeted specifically for sampling. The probability of having an antibody against a human strain was higher in dogs with clinical illness than in healthy dogs. Especially for H1N1, the risk was observed to be significantly higher in dogs with respiratory-related illness. It could be inferred that the risk of viral exposure of dog from human is higher under conditions that result in some clinical respiratory presentation. However, it is difficult to conclude whether there was a causal relationship between the observed prevalence and the history of respiratory illness, or if it was a coincidence due to unknown factors; for example, the quality of care of individual dogs, such as variation in vaccination and/or housing conditions. However, we cannot completely rule out the possible causal relationship of this finding since H1N1/2009 was confirmed to cause mild respiratory illness under experimental conditions [21].

The dog's age was another risk factor analyzed in our study. In previous studies, the prevalence of a novel canine strain was highest during the age range of 2 to 5 years [31]. In our study, seropositivity was observed only in sera collected from dogs $2 \leq \text{age} < 14$ years old. When collected from dogs older than 14 years old, all sera were seronegative to CIV; contrasting with human flu strains which showing the highest prevalence in that age group. This discrepancy may be explained by the difference in exposure conditions for CIV and human strains, which appear to be occasional from dogs for the former, but to be continuous from human for the latter. The negative influenza antibody status of dogs younger than 2 years old was unexpected since all ages of dogs are reported to be susceptible to CIV [7,9,16], and young pups can be especially vulnerable to the infectious disease [7]. Consistent with our results, a study conducted in Colorado showed a 0% prevalence in dogs younger than 2 years old [5], and a study conducted on dogs with flu-like syndrome showed a significantly low risk of having antibody against CIV in dogs less than 1 year old [4]. On the other hand, a study conducted in China reported a different finding in which the prevalence in 0- to 2-year-old dogs was

similar to the overall prevalence. Taken together, the chance of exposure of young pups to CIV appears to be lower than, or at most similar to, that of adult dogs.

Since all sera used in this study were passively collected from veterinary hospitals, we could not avoid several study limitations. First, most dogs in this study were involved in clinical cases and control selection bias may have resulted in higher exposure frequencies and other risk factors in hospitalized individuals compared to those in a normal reference population. Second, the access to information on dogs was limited and diverse risk factors such as exposure to dog day care centers, endemic areas, and vaccine status could not be evaluated. With regard to vaccine status, we have assumed that the dogs presented for wellness checks were almost assuredly not vaccinated since The Ohio State University Veterinary Teaching Hospital Pharmacy did not stock the H3N8 vaccine during the study period. Also, flu vaccination for dogs was not a common procedure at the time of sample collection and the American Animal Hospital Association (AAHA) canine vaccine guidelines [2] does not include CIV H3N8 vaccine as a core vaccine. Thus, we expect that the CIV H3N8 antibody detected in this study was mostly induced by natural infection.

In conclusion, based on the low prevalence of CIV H3N8, we infer that the CIV has not yet actively established itself in Ohio; thus, the risk of Ohio becoming an endemic area could be reduced by implementing proper control measures. Since the prevalences of both H1N1 and H3N2 suggest that human-to-dog exposure of the influenza virus is ongoing, and considering the risk of reassortment of different flu viruses in canine species, the zoonotic importance of canine species in influenza ecology should not be overlooked. Our results warrant continuing efforts in investigating flu activity in general dog populations, further identification and verification of risk factors, validation of diagnostic tests, and development of active control measures.

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Conflict of Interest

The authors declare no conflicts of interest.

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